Molecular Modelling of Adenosine Receptors and Halogen Bonds

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Summary

G-protein coupled receptors (GPCRs) are membrane proteins regulating cell's life by response to a wide variety of physical-chemical stimuli, either endogenous or exogenous, and represent a valuable biological target for the therapeutic treatment of several diseases. Since 2000, when the first GPCR belonging to the class A (bovine rhodopsin) was crystallized and solved using X-ray diffraction, a new way was paved for the structure-based rational design of new powerful drugs by computational methods. Among the class A GPCR family members, the human adenosine receptors (hARs) are interesting pharmacological targets due to: the related amount of well solved crystal structures bound to agonists and antagonists; the existence of receptor subtypes differently distributed in the human body; their role in many physio-pathological conditions. Molecular Mechanics (MM) forcefields are generally suitable for systems made up of thousands or more atoms, thus, they were extensively used in our work. However, dealing with the ligand-protein recognition mechanisms may sometimes require estimations at a lower dimensional scale, which should include electrons explicitly, i.e. it could require Quantum Mechanics (QM) performances. This represents a suitable case for halogen bonds (XBs). Most of the approved drugs contain at least a halogen atom, commonly considered crucial in conferring both efficacy and selectivity towards their biological targets. Unfortunately, it is not yet possible to model accurately this kind of interaction as occurring in ligand-protein systems.

The present Ph.D. work tries to give new insights into how the drug design process could be improved in order to achieve target efficacy and selectivity at different levels of computational accuracy, starting from the fundamentals reported in *section 1* and using tools described in *section 2*. With this in mind, we addressed the structural complexity as well as the homology of hARs using molecular mechanics methods and a novel correlation coefficient, called EM, implemented in our group for the comparative analysis of data (*section 2.3*).

Section 3.1. By means of molecular docking and EM analysis, we demonstrated that inclusion of sodium ion and its first hydration sphere into docking simulations of 15

antagonists and 36 A_{2A} AR crystal structures improves significantly either precision or accuracy of ligand posing, regardless of the nature of the ligand and of the docking protocol, indicating that such structural elements should be incorporated in any molecular docking study on orthosteric antagonists.

Section 3.2. Basing on structural comparison, docking simulations and EM coefficient analysis of hA_3 active/inactive AR models, derived by hA_1 and hA_{2A} AR crystal structures, it was found that the hA_1 template is a valid alternative to the conventionally used hA_{2A} AR templates.

Sections 3.3-3.5. Ligand structure-activity relationships and selectivity towards the hA_3 AR subtype were explored by means of either experimental or computational studies (docking, molecular dynamics) on synthetized compounds. The latter were designed in order to be also conjugated with fluorescent probes. Affinity tests showed a variegated binding profile of antagonists, indicating a strong selectivity in favour of the hA_3 AR as compared to the hA_1 subtype; in general, the best affinity has been observed for A_3 and A_{2A} receptors. The role of ligand scaffold, linker and probes was elucidated. In *sections 3.4-3.5.* we used the inactive A_3 AR homology model described in *section 3.2.*, moreover, sodium ion and its first hydration sphere were included in both molecular docking and molecular dynamics simulations. Peculiar residues were identified by molecular docking and SuMD (Supervised Molecular Dynamics) simulations as important for either affinity or selectivity profiles observed, respectively located at the orthosteric site and extracellular loops of any receptor subtype.

In *section 3.6.* we addressed the close mechanism of XB interactions in ligand-protein models, by means of the Kohn-Sham Molecular Orbital (KS MO) theory and of Energy Decomposition Analysis (EDA). We found that peptide methyl and amino building blocks increased the stability of XB complexes by electrostatics, dispersion and charge transfer from the Lewis base to the halogenated Lewis acid; on the contrary, the inclusion of a peptidic carbonyl, adjacent to the XB pair, lowered it. Geometrical descriptors were evaluated and the dihedral angle φ resulted to be more representative of the chemical environment than the commonly used angle θ. A comparison between hydrogen bonds (HBs) and XBs was performed. HBs showed better energies than XBs because of a lower Pauli repulsion for the same bond lengths. Finally, the interaction

energy of pyridine-bromobenzene complex was estimated for perpendicular XB geometries, as function of either the *azimuthal* or *zenith* orientations: results were in total contrast with “σ-hole” theory predictions, because electrostatics were even improved at 90° θ ($C_{Ar}BrO$) angles. Indeed, the total interaction energy resulted destabilizing, as a consequence of the Pauli repulsion arising from the increased monomers’ HOMO-HOMO overlap.

In summary, we used a combination of strategies, based mainly on MM, QM and mathematical statistics, in order to study a wide variety of cases, either purely computational or experimental. The EM correlation coefficient was introduced and extensively exploited for data analysis. Ranging from the adenosine receptor structural complexity and its ligands to the mechanism of halogen bonds, we provided a new understanding of the ligand-protein interactions from different perspectives. We laid the foundation for the more realistic and accurate description of molecular phenomena, which could finally fulfill the gap between experimental and computational observations, as well as the gap in molecular mechanics and quantum mechanics applications.